

addition-present post-combination affinity reagent and an unbound remainder;

separating each said addition present post-combination affinity reagent from each of said unbound remainder to form at least one isolated addition-present post-combination affinity reagent;

adding a laser desorption/ionization agent to each said isolated addition-present post-combination affinity reagent to form an addition-present mass spectrometric mixture therewith;

mass spectrometrically analyzing each said addition-present mass spectrometric mixture to produce an addition-present mass spectrum having an internal reference species mass spectrometric response located at the unique mass-to-charge ratio of said internal reference species, and an addition-present mass spectrometric response located at the unique mass-to-charge ratios of each said certain antigen;

normalizing each said addition-present mass spectrum and each said addition-free mass spectrum with the respected said internal reference species mass spectrometric response to produce an addition-free normalized antigen mass spectrometric response for the addition-free sample, and addition-present normalized mass spectrometric response for each said addition-present sample;

determining a set of changes between said addition-free normalized antigen mass spectrometric response and each said addition-present normalized antigen mass spectrometric response for each said certain antigen by subtracting said addition-free normalized antigen mass spectrometric response from each said addition-present normalized antigen mass spectrometric response for each said certain antigen in each said addition-present sample;

determining the relationship between said set of changes and each of the corresponding changes in concentration for each said standard addition antigen in each said addition-present sample resulting from the addition of said standard addition antigen preparation; and

quantifying each said certain antigen detected in said specimen using said addition-free and addition-present normalized mass spectrometric responses and said determined relationship.

6. The method of claim 5 further including the steps of adding a disassociation agent to said isolated addition-free affinity reagent and to each of said isolated addition-present affinity reagents prior to said adding laser desorption/ionization agent step.

7. The method of claim 4, further including the steps of:

making a standard addition preparation, containing a known concentration of each said certain antigen or a counterpart thereof;

immobilizing at least one antibody onto a solid substrate to produce said affinity reagent;

combining an effective amount of said affinity reagent with said specimen to produce an addition-free post-combination affinity reagent and a first unbound remainder, said first unbound remainder containing the majority of each said certain antigen;

separating said addition-free post-combination affinity reagent from said first unbound remainder to form an isolated addition-free post-combination affinity reagent;

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adding a laser desorption/ionization agent to said isolated addition-free post-combination affinity reagent to form an addition-free mass spectrometric mixture;

mass spectrometrically analyzing said addition-free mass spectrometric mixture to produce an addition-free mass spectrum having an internal reference species mass spectrometric response located at the unique mass-to-charge ratio of said internal reference species, and an addition-free mass spectrometric response at the unique mass-to-charge ratio of each said certain antigen present in said specimen;

adding a known quantity of said standard addition preparation to said first unbound remainder to produce an addition-present first unbound remainder in which the concentration of each said certain antigen or said counterpart has been increased by a known amount;

combining an effective amount of said affinity reagent with said addition-present first unbound remainder to produce a addition-present post-combination affinity reagent and a second unbound remainder, said second unbound remainder containing the majority of each said certain antigen or said counterpart;

separating said addition-present post-combination affinity reagent from said second unbound remainder to form an isolated addition-present post-combination affinity reagent;

adding a laser desorption/ionization agent to said isolated addition-present post-combination affinity reagent to form a addition-present mass spectrometric mixture;

mass spectrometrically analyzing said addition-present mass spectrometric mixture to produce a addition-present mass spectrum having an internal reference species mass spectrometric response

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located at the unique mass-to-charge ratio of said internal reference species, and an addition-present mass spectrometric response at the unique mass-to-charge ratio of each said certain antigen when said certain antigen is present in said specimen;

normalizing each said addition-present antigen mass spectrum and each said addition-free mass spectrum with the respective said internal reference species mass spectrometric response to produce an addition-free normalized antigen mass spectrometric response for the addition-free sample and an addition-present normalized mass spectrometric response for each said addition-present sample;

determining a set of changes between each said addition-free normalized antigen mass spectrometric response and each said addition-present normalized antigen mass spectrometric response for each said antigen by subtracting said addition-free normalized antigen mass spectrometric response from each said addition-present normalized antigen mass spectrometric response for each said antigen in each said addition-present sample;

determining the relationship between each said set of changes and each of the corresponding changes in concentration of standard addition in each said addition-present sample resulting from the addition of said standard addition antigen preparation; and

quantifying each said certain antigen detected in said specimen using said addition-free and addition-present normalized antigen mass spectrometric responses and said determined relationship.

8. The method of claim 7 further including the step of adding a disassociation agent to each said isolated addition-free post-combination affinity reagent, and to each said isolated addition-present post-combination affinity reagent prior to said adding laser desorption/ionization agent step.

the concentration of said preparation antigen is varied between said plurality of preparations in known amounts;

adding sufficient said internal reference species to each said preparation so that the concentration of said internal reference species is the same or is known for each said preparation and said specimen;

combining an effective amount of said affinity reagent to each of said preparations to produce a post-combination preparation affinity reagent and an unbound preparation remainder;

separating each said post-combination preparation affinity reagent from said unbound preparation remainder to form isolated post-combination preparation affinity reagents;

adding a laser desorption/ionization agent to each said isolated post-combination preparation affinity reagent to form preparation mass spectrometric mixtures;

mass spectrometrically analyzing each said preparation mass spectrometric mixture, each mass spectrometric analysis to produce a preparation mass spectrum, each of said preparation mass spectra containing a mass spectrometric response for said internal reference species located at the unique mass-to-charge ratio of said internal reference species and a preparation antigen mass spectrometric response for each said preparation antigen present in said preparation mass spectrometric mixture, said preparation antigen mass spectrometric response being located at the unique mass-to-charge ratio of the relevant said preparation antigen;

normalizing said specimen mass spectrum and each of said preparation mass spectra to the mass spectrometric response obtained for each said internal reference species to obtain a

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internal reference species and each of said certain antigen species that is present in said specimen to produce a post-combination affinity reagent and an unbound remainder;

separating said post-combination affinity reagent from said unbound remainder to form an isolated post-combination affinity reagent;

adding a laser desorption/ionization agent to said isolated post-combination affinity reagent to form a mass spectrometric mixture;

mass spectrometrically analyzing said mass spectrometric mixture to produce a mass spectrum, said mass spectrum indicating whether said specimen contained any of said certain antigen species by exhibiting an antigen species mass spectrometric response located at the unique mass-to-charge ratio of each of said certain antigen species, said mass spectrum also having a mass spectrometric response for each of said internal reference species; and

interpolating each said antigen species mass spectrometric response to the internal reference species mass spectrometric response immediately above and below in magnitude of each of said antigen species mass spectrometric response to quantify each said certain antigen species in said specimen.

12. The method of claim 11 further including the step of adding a disassociation agent to said isolated post-combination affinity reagent prior to said adding laser desorption/ionization agent step.

13. The method of claim 4, further including the steps of:

making a single reference sample containing a known concentration of a reference antigen for each said certain antigen wherein each said reference antigen is said certain antigen or a counterpart thereof;

adding said internal reference species to said reference sample such that the ratio of the concentration of said internal reference species in said reference sample to the concentration of said internal reference species in said specimen is known;

immobilizing at least one antibody on a solid substrate to produce said affinity reagent;

combining an effective amount of said affinity reagent with said specimen to produce a specimen post-combination affinity reagent and an unbound remainder;

separating said specimen post-combination affinity reagent from said unbound remainder to form an isolated specimen post-combination affinity reagent;

adding a laser desorption/ionization agent to said isolated specimen post-combination affinity reagent to form a specimen mass spectrometric mixture;

mass spectrometrically analyzing said specimen mass spectrometric mixture to produce a specimen mass spectrum having a mass spectrometric response for each of said certain antigen species present in said specimen located at the respective unique mass-to-charge ratio of each said certain antigen species, and a specimen internal reference species mass spectrometric response located at the unique mass-to-charge ratio of said internal reference species;

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contained each said certain antibody species by exhibiting a mass spectral signal located at the unique mass-to-charge ratio of each said certain antibody species.

17. The method of claim 16, wherein said antigen is an allergen.

18. The method of claim 16, further including the step of adding a disassociation agent to each said isolated post-combination affinity reagent prior to said adding laser desorption/ionization agent step.

19. The method of claim 18, wherein said antigen is an allergen.

20. A method for determining the amount of a certain antibody present in a specimen, comprising the steps of:

adding an internal reference species to said specimen when said specimen does not already contain one;

means for capturing and isolating said certain antibody and said internal reference species from said specimen, said capturing and isolating means involving the use of an affinity reagent having a specific affinity for said certain antibody and said internal reference species; and

means for quantifying said certain antibody, wherein said quantifying means involves mass spectrometric analysis of said isolated certain antibody and said internal reference species.

21. The method of claim 20, further comprising the steps of:

making at least one standard addition preparation, each containing a known concentration of said certain antibody or a counterpart of said certain antibody;

dividing said specimen to form a first divided sample and at least one remainder sample;

adding a known amount of said certain antibody or said counterpart of said certain antibody to each said remainder sample to produce at least one addition-present sample wherein the increase in concentration of said added certain antibody or said counterpart is known;

immobilizing at least one affinant onto a solid substrate to produce said affinity reagent;

combining an effective amount of said affinity reagent with said first divided sample to produce an addition-free post-combination affinity reagent and a first unbound remainder;

separating said addition-free affinity reagent from said first unbound remainder to form an isolated addition-free post-combination affinity reagent;

adding a laser desorption/ionization agent to said isolated addition-free post-combination affinity reagent to form an addition-free mass spectrometric mixture;

mass spectrometrically analyzing said addition-free mass spectrometric mixture to produce an addition-free mass spectrum having an internal reference species mass spectrometric response at the unique mass-to-charge ratio of said internal reference species, and an addition-free antibody mass spectrometric response at the unique mass-to-charge ratio of said certain antibody present in said specimen;

combining an effective amount of said affinity reagent with each said addition-present samples each combination producing an

addition-present post-combination affinity reagent and an unbound remainder;

separating each said addition-present post-combination affinity reagent from each said unbound remainder to form at least one isolated addition-present post-combination affinity reagent;

adding a laser desorption/ionization agent to each of said isolated addition-present post-combination affinity reagent to form at least one addition-present mass spectrometric mixture therewith;

mass spectrometrically analyzing each said addition-present mass spectrometric mixture to produce an addition-present mass spectrum having an internal reference species mass spectrometric response located at the unique mass-to-charge ratio of said internal reference species, and an addition-present antibody mass spectrometric response located at the unique mass-to-charge ratio of said certain antibody;

normalizing each said addition-present antibody mass spectrum and each said addition-free antibody mass spectrum with the respective said internal reference species to produce an addition-free normalized antibody mass spectrometric response for said addition-free sample, and an addition-present normalized antibody mass spectrometric response for each said addition-present sample;

determining a set of changes between said addition-free normalized antibody mass spectrometric response and each said addition-present normalized antibody mass spectrometric response for said certain antibody species by subtracting said addition-free normalized antibody mass spectrometric response from each said addition-present normalized antibody mass spectrometric response for each of said addition-present sample;

determining the relationship between said set of changes and each of the corresponding changes in concentration of said standard addition antibody in each said addition-present sample resulting from the addition of said standard addition antibody preparation; and

quantifying said certain antibody detected in said specimen using said addition-free and addition-present normalized antibody mass spectrometric responses and said determined relationship.

22. The method of claim 21 further including the steps of adding a disassociation agent to said isolated addition-free affinity reagent and each of said isolated post-combination addition-present affinity reagents prior to said adding laser desorption/ionization agent step.

23. The method of claim 20, further including the steps of:

making a standard addition preparation, containing a known concentration of a standard addition antibody, said standard addition antibody being said certain antibody or a counterpart thereof;

immobilizing at least one affinant onto a solid substrate to produce said affinity reagent;

combining an effective amount of said affinity reagent with said specimen to produce an addition-free post-combination affinity reagent and a first unbound remainder, said first unbound remainder containing the majority of said certain antibody;

separating said addition-free post-combination affinity reagent from said first unbound remainder to form an isolated addition-free post-combination affinity reagent;

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located at the unique mass-to-charge ratio of said internal reference species and an addition-present antibody mass spectrometric response at the mass-to-charge ratio of said certain antibody when said certain antibody is present in said specimen;

normalizing each said addition-present antibody mass spectrum and said addition-free antibody mass spectrum with the respective said internal reference species mass spectrometric response to produce an addition-free normalized antibody mass spectrometric response for the addition-free sample and an addition-present normalized antibody mass spectrometric response for each said addition-present sample;

determining a set of changes between said addition-free normalized antibody mass spectrometric response and each said addition-present normalized antibody mass spectrometric response for said certain antibody by subtracting said addition-free normalized antibody mass spectrometric response from each said addition-present normalized antibody mass spectrometric response for said certain antigen in each said addition-present sample;

determining the relationship between said set of changes and each of the corresponding changes in concentration of standard addition in each said addition-present sample resulting from the addition of said standard addition antibody preparation; and

quantifying said certain antibody detected in said specimen using said addition-free and addition-present normalized antibody mass spectrometric responses and said determined relationship.

24. The method of claim 23 further including the step of adding a disassociation agent to said isolated addition-free post-combination affinity reagent, and each said isolated addition-present post-combination affinity reagent prior to said adding laser desorption/ionization agent step.

determining the mathematical relationship between said normalized preparation antibody mass spectrometric responses and said preparation antibody concentrations;

locating a point on said mathematical relationship which appertains to said normalized certain antibody mass spectral response; and

determining the concentration that corresponds to said point, thereby quantifying said certain antibody.

26. The method of claim 25 further including the step of adding a disassociation agent to said isolated post-combination affinity reagent prior to said adding laser desorption/ionization agent step.

27. The method of claim 20, further including the steps of:

preparing a single reference sample containing a known concentration of a reference antibody, wherein said reference antibody is said certain antibody or a counterpart thereof;

immobilizing at least one affinant on a solid substrate to produce said affinity reagent;

combining an effective amount of said affinity reagent with said specimen to produce a specimen post-combination affinity reagent and a first unbound remainder;

separating said specimen post-combination affinity reagent from said first unbound remainder to form a first isolated specimen post-combination affinity reagent;

adding a laser desorption/ionization agent to said first isolated specimen post-combination affinity reagent to form a specimen mass spectrometric mixture;

mass spectrometrically analyzing said specimen mass spectrometric mixture to produce a specimen mass spectrum having a mass spectrometric response for said certain antibody at the mass-to-charge ratio of said certain antibody, and also having a specimen internal reference species mass spectrometric response located at the mass-to-charge ratio of said internal reference species;

combining an effective amount of said affinity reagent with said single reference sample to produce a reference sample post-combination affinity reagent and a second unbound remainder;

separating said reference sample post-combination affinity reagent from said second unbound remainder to form an isolated reference sample post-combination affinity reagent;

adding a laser desorption/ionization agent to said isolated reference sample post-combination affinity reagent to form a single reference sample mass spectrometric mixture;

mass spectrometrically analyzing said single reference sample mass spectrometric mixture to produce a single reference sample mass spectrum having a mass spectrometric response for said reference antibody located at the unique mass-to-charge ratio of said reference antibody, and a second internal reference species mass spectrometric response located at the mass-to-charge ratio of said internal reference species;

normalizing said specimen mass spectrum and said single reference sample mass spectrum using said first and second internal reference species mass spectrometric responses; and

equating the ratio of the magnitudes of said certain antibody mass spectrometric response and said reference antibody mass spectrometric response to the ratio of the known concentration of said counterpart antibody and the unknown concentration of said certain antibody to determine the concentration of said certain antibody.

28. The method of claim 27 further comprising the step of adding a disassociation agent to each of said first and second isolated affinity reagent prior to said adding laser desorption/ionization agent step.

29. A method of simultaneously determining whether an antibody population contained in a specimen contains one or more certain antibody species, comprising the steps of:

combining said specimen with a solid substrate having a non-specific affinity for antibodies to immobilize said antibody population onto said solid substrate and produce an affinity reagent;

placing said affinity reagent into a preparation containing at least one known antigen, each said known antigen having a specific affinity for one of said certain antibodies to produce a post-combination affinity reagent and a screened preparation;

separating said post-combination affinity reagent from said screened preparation to form an isolated post-combination affinity reagent;

adding a laser desorption/ionization agent to said isolated post-combination affinity reagent to form a mass spectrometric mixture; and

mass spectrometrically analyzing said mass spectrometric mixture to produce a mass spectrum, said mass spectrum indicating whether said antiserum contained each of said certain antibody species by exhibiting a mass spectrometric response located at the unique mass-to-charge ratio of the relevant said known antigen which has a specific affinity for that said certain antibody species.

30. The method of claim 29 further including the step of adding a disassociation agent to said isolated post-combination affinity reagent prior to said adding laser desorption/ionization agent step.

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